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Callus Induction in Seedling-Derived Explants of *Erythrina Variegata* L.: Effects of Basal Medium and Plant Growth Regulators

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Abstract: ***Callus induction in Erythrina variegata L. was examined using seedling-derived explants cultured on different basal media and plant growth regulator (PGR) treatments. Hypocotyl, internode, root, cotyledon and leaf segments were initially cultured on Murashige and Skoog (MS), Gamborg B_5 and woody plant medium (WPM), each supplemented with 2.0 mg L^{-1} indole-3-butyric acid (IBA). Hypocotyls were the most responsive explants. On MS medium, they showed 90% callus response and a mean fresh weight of 1060 ± 437.0 mg; corresponding responses on B_5 and WPM were 75% and 50%, respectively. Internode segments showed lower responses, while root, cotyledon and leaf explants did not produce callus in this initial comparison. In the PGR screen, 2.0 mg L^{-1} IBA produced the highest recorded hypocotyl response (90%), followed by 2.0 mg L^{-1} IAA, NAA and picloram (60% each). A combination of 5.0 mg L^{-1} 6-benzylaminopurine (BAP) and 0.5 mg L^{-1} NAA also produced 90% response and green, friable callus. The results identify seedling hypocotyls on MS medium with IBA as the most responsive treatment among those tested. The experiments document callus induction but do not establish a reproducible callus-to-shoot or callus-to-embryo regeneration pathway.***

Keywords: *Erythrina variegata*; callus induction; hypocotyl; Indole-3-butyric acid.

1. Introduction

Erythrina variegata L. (Fabaceae), commonly known as the Indian coral tree (*Badida Chettu*, *Badachipa Chettu* in Telugu), is a multipurpose tropical tree cultivated for ornamental planting, shade, live fencing and agroforestry uses. As with other woody legumes, the species is of interest for in vitro culture because controlled culture systems can provide uniform plant material and allow the responses of defined tissues to be examined under standardized conditions. The success of in vitro culture is influenced by the physiological age and identity of the explant, basal-medium composition, the concentration and balance of plant growth regulators, and the culture environment.

Callus induction is frequently used as an experimental stage in plant cell and tissue culture. Callus cultures can provide material for studies of cellular differentiation, organogenesis, somatic embryogenesis and secondary-metabolite production. However, callus formation alone does not demonstrate morphogenic competence. A callus may remain non-organogenic, and the ability to produce shoots or embryos must be established through separate, documented regeneration experiments. This distinction is especially important in woody species, in which callus induction and subsequent shoot differentiation may respond differently to changes in auxin and cytokinin balance.

Previous work has documented several in vitro responses in *E. variegata*. Shasthree *et al*. [11] reported multiple-shoot formation from cotyledon and leaf explants of in vitro-grown seedlings. Shasthree *et al*. [10] described plantlet regeneration by organogenesis from leaf- and stem-derived cultures, using BAP, kinetin, thidiazuron (TDZ) and coconut milk in different culture combinations. In a separate study, Shasthree *et al*. [9] reported in vitro rooting from callus cultures derived from seedling explants and described rhizogenesis as more frequent than caulogenesis under the conditions tested. These reports show that different explants and PGR regimes can produce distinct developmental responses in this species.

Javed and Anis [3] subsequently developed a nodal-explant micropropagation system for *E. variegata*, with shoot multiplication optimized using benzyladenine and NAA. Javed *et al*. [5] investigated copper sulphate for reducing latent microbial contamination in *E. variegata* cultures, while Javed *et al*. [4] developed synthetic-seed procedures using encapsulated nodal segments. These studies provide additional species-specific context for in vitro propagation. Carlini *et al*.

[1] evaluated seed scarification and in vitro callus-culture methods in *E. verna* and *E. speciosa*; their findings are relevant at the genus level but cannot be assumed to apply directly to *E. variegata* because tissue-culture responses are species- and explant-dependent.

Callus-mediated plantlet production has also been demonstrated in other woody legumes. For example, Sankara Rao [8] obtained shoot buds from somatic callus tissue of *Dalbergia latifolia* using sequential changes in the balance of benzyladenine and NAA. This provides comparative methodological context rather than direct evidence for *Erythrina*. In view of the variation reported among species and explant sources, a comparative evaluation of seedling-derived tissues and culture media is useful for determining which combinations favour callus induction in *E. variegata*.

The present study evaluated callus induction from hypocotyl, internode, root, cotyledon and leaf explants of seed-derived *E. variegata* seedlings. The influence of MS, B_5 and WPM basal media was examined, followed by screening of selected auxins and cytokinin-containing combinations. The objective was to identify the explant–medium–PGR treatments associated with the highest callus response and callus fresh weight, while distinguishing callus induction from subsequent plant regeneration.

2. Materials and Methods

2.1 Plant material, seed collection and seedling establishment

Mature fruits of *Erythrina variegata* were collected during May–June from Bakarapet (13.663329°N, 79.157599°E; 602 m a.s.l.), Annamayya District (it was Chittoor District), Andhra Pradesh, India. A voucher specimen (FAP34270) is deposited in the herbarium of Sri Krishnadevaraya University (SKU). The fruits were shade-dried and the seeds were separated for germination. Aseptic seedlings raised from these seeds were used as the source of juvenile explants for the callus experiments (**Plate 1**).

For in vitro establishment, mechanically separated seeds were washed in 0.1% Laboline for 5 min and rinsed under running tap water for 20 min. The seeds were scarified with 98% sulphuric acid (H_2SO_4) for 2 min to soften the hard seed coat and then washed thoroughly under running tap water for 20 min. Surface sterilization was carried out sequentially with 0.1% mercuric chloride ($HgCl_2$) for 2 min, followed by 90% ethanol for 30 s. The seeds were then rinsed thoroughly with sterile double-distilled water before inoculation onto culture medium.

2.2 Basal media and culture conditions

The basal media used for callus induction were Murashige and Skoog (MS) medium [7], Gamborg B_5 medium [2] and woody plant medium (WPM) [6]. Media were prepared using the respective published formulations. Sucrose was supplied at 2% (w/v) and bacteriological-grade agar at 0.8% (w/v). The medium pH was adjusted to 5.6–5.8 before autoclaving at 121 °C and 15 lb in^{-2} for 15 min. Culture vessels were maintained at 25 ± 2 °C under a 16-h photoperiod provided by cool-white fluorescent lamps; the recorded illumination was approximately 2000 lux.

2.3 Explant and basal-medium evaluation

Hypocotyl, internode, root, cotyledon and leaf segments excised from aseptic seedlings were cultured on MS, B_5 and WPM media supplemented with 2.0 mg L^{-1} IBA. The response was evaluated as the percentage of explants producing callus and, for responding treatments, fresh callus weight. Callus colour and texture were recorded descriptively. Fresh callus weights are presented as mean ± standard deviation (SD), as reported in the experimental **Table 1**.

2.4 Screening of plant growth regulators

Further callus-induction experiments were conducted using hypocotyl and internode explants on MS medium supplemented with selected auxins. The PGRs evaluated were 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), IBA, 1-naphthaleneacetic acid (NAA), picloram and dicamba at the concentrations reported in Table 2. Percentage response, degree of callusing and callus appearance were recorded. The summary in **Table 2** presents the highest hypocotyl response reported for each regulator and the corresponding internode response at that concentration.

2.5 BAP combinations and growth additives

Hypocotyl explants were also cultured on MS medium supplemented with BAP in combination with NAA, IAA, IBA, 2,4-D, silver nitrate ($AgNO_3$), casein hydrolysate or coconut milk. Response percentage and qualitative callus characters (colour and texture) were recorded (**Table 3**).

2.6 Data recording

The experiments were repeated three times with 15 replicates per treatment, as stated in the original experimental table footnotes. Results are presented descriptively. No inferential statistical comparisons are reported.

3. Results

3.1 Effect of explant type and basal medium on callus induction

Callus induction varied among explant types and basal media. Hypocotyl segments showed the highest response on all three media tested. On MS medium containing 2.0 mg L^{-1} IBA, hypocotyl explants produced 90% callus response and a fresh callus weight of 1060 ± 437.0 mg. The response on B_5 was 75%, with 831.2 ± 354.06 mg fresh weight, whereas WPM supported 50% response and 316.8 ± 351.8 mg. Internode segments showed lower responses than hypocotyls, with the highest

response (70%) and fresh weight (544 ± 375.9 mg) also recorded on MS medium. Root, cotyledon and leaf explants showed no recorded callus response under the same initial screening conditions (**Table 1 & Fig. 1**).

Table 1: Callus induction response and fresh callus weight from different seedling explants on MS, B_5 and WPM media supplemented with 2.0 mg L^{-1} IBA. NR, no response.

Explant	Basal medium	PGR	Response (%)	Fresh callus weight (mg; mean ± SD)
Hypocotyl	MS	2.0 mg L^{-1} IBA	90	1060 ± 437.0
Hypocotyl	B_5	2.0 mg L^{-1} IBA	75	831.2 ± 354.06
Hypocotyl	WPM	2.0 mg L^{-1} IBA	50	316.8 ± 351.8
Internode	MS	2.0 mg L^{-1} IBA	70	544 ± 375.9
Internode	B_5	2.0 mg L^{-1} IBA	60	396.1 ± 360.7
Internode	WPM	2.0 mg L^{-1} IBA	50	161.06 ± 187.2
Root	MS/B_5/ WPM	2.0 mg L^{-1} IBA	NR	—
Cotyledon	MS/B_5/ WPM	2.0 mg L^{-1} IBA	NR	—
Leaf	MS/B_5/ WPM	2.0 mg L^{-1} IBA	NR	—

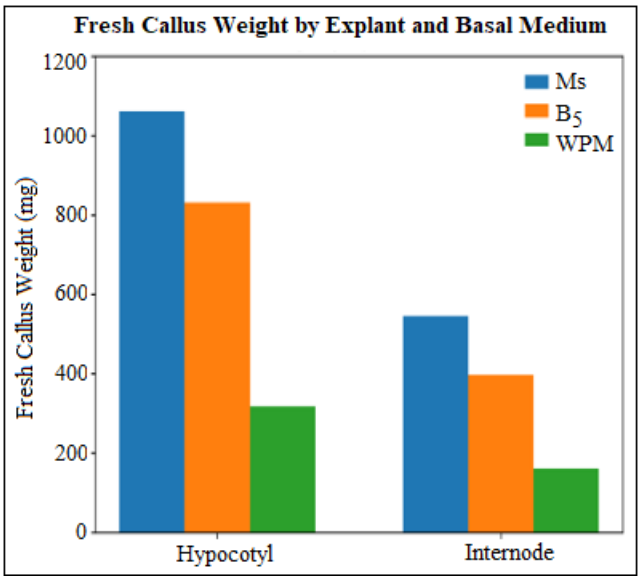


Figure 1: Fresh callus weight recorded from hypocotyl and internode explants on MS, B_5 and WPM media containing 2.0 mg L^{-1} IBA

3.2 Response to individual plant growth regulators

The response to the PGR screen differed between hypocotyl and internode explants. IBA at 2.0 mg L^{-1} produced the highest reported hypocotyl response (90%) and an internode response of 70% at the same concentration. IAA and NAA at 2.0 mg L^{-1} each produced 60% response in hypocotyl explants and 45% in internode explants. Picloram at 2.0 mg L^{-1} produced 60% response in hypocotyls and 50% in internodes. The highest reported response with dicamba was 40% in hypocotyls at 3.0 mg L^{-1}. The 2,4-D treatment showed a maximum reported hypocotyl response of 35% at 3.0 mg L^{-1}. Callus appearance varied from green and compact to yellowish-white or brown and friable (**Table 2, Plate 2 & Plate 3**).

Table 2: Summary of selected PGR responses in hypocotyl and internode explants.

PGR	Concentration with highest reported hypocotyl response (mg L^{-1})	Hypocotyl response (%)	Internode response at same concentration (%)	Recorded callus character
2,4-D	3.0	35	20	Green, compact/friable; variable
IAA	2.0	60	45	Yellow-brown, compact / yellow-green, friable
IBA	2.0	90	70	Yellowish-white, compact/friable
NAA	2.0	60	45	Yellowish-white or green, compact
Picloram	2.0	60	50	Brown, compact
Dicamba	3.0	40	30	Green or yellowish-brown, friable

3.3 Callus response to BAP combinations and growth additives

Hypocotyl explants cultured on MS medium containing 5.0 mg L^{-1} BAP and different NAA concentrations showed response values ranging from 65% to 90%. The highest response (90%) was recorded with 0.5 mg L^{-1} NAA, and the callus was described as green and friable, with greater callus production. The 5.0 mg L^{-1} BAP + 0.3 mg L^{-1} NAA treatment produced 85% response. BAP–$AgNO_3$ combinations produced 60–75% response, with light-green friable callus. Other BAP combinations containing IAA, IBA, 2,4-D, casein hydrolysate or coconut milk produced variable response percentages (**Table 3 & Plate 3**).

Table 3: Callus response to BAP combinations and selected growth additives in hypocotyl explants on MS medium

Treatment (mg L^{-1} unless stated)	Response (%)	Nature of callus
5.0 BAP + 0.1 NAA	65	Green, hard
5.0 BAP + 0.2 NAA	80	Green, friable
5.0 BAP + 0.3 NAA	85	Green, friable
5.0 BAP + 0.4 NAA	70	Green, friable
5.0 BAP + 0.5 NAA	90	Green, friable; more callus

5.0 BAP + 0.1 $AgNO_3$	75	Light-green, friable
5.0 BAP + 0.2 $AgNO_3$	70	Light-green, friable
5.0 BAP + 0.3 $AgNO_3$	60	Light-green, friable
3.0 BAP + 0.1 NAA	50	Yellowish-white, friable
3.0 BAP + 0.2 NAA	40	Yellowish-white, friable
3.0 BAP + 0.1 IAA	55	Green, compact; less
3.0 BAP + 0.2 IAA	60	Light-green, compact
3.0 BAP + 0.1 IBA	70	Yellowish-white, friable
3.0 BAP + 0.2 IBA	75	Yellowish-white, friable
3.0 BAP + 0.1 2,4-D	55	Green, friable; less
3.0 BAP + 0.2 2,4-D	45	Green, friable; less
3.0 BAP + 0.5% casein hydrolysate	40	Yellowish-green, friable
5.0 BAP + 15% coconut milk	45	Green, friable

4. Discussion

4.1 Explant dependence of callus induction

The results identify seedling hypocotyls as the most responsive explant source for callus induction among the tissues evaluated. Hypocotyls produced higher response percentages and fresh callus weights than internode segments, while root, cotyledon and leaf explants did not respond in the initial comparison on the three basal media containing 2.0 mg L^{-1} IBA. These observations indicate that tissue identity and developmental state influenced callus formation under the tested conditions. The explants were obtained from aseptic seedlings and therefore represented juvenile material. Hypocotyls are readily obtained from seedlings of a relatively uniform developmental age, which may contribute to their usefulness in in vitro manipulation.

The response of *E. variegata* is consistent with the broader observation that morphogenic responses in this species depend on explant source and culture regime. Shasthree *et al*. [11] reported multiple-shoot formation from cotyledon and leaf explants, while Shasthree *et al*. [10] reported organogenic plantlet regeneration from leaf- and stem-derived cultures. The absence of callus in the present initial comparison should therefore be interpreted in relation to the particular medium and IBA concentration tested, rather than as evidence that root, cotyledon or leaf tissues are intrinsically incapable of responding in vitro.

4.2 Influence of basal-medium composition

MS medium supported the highest recorded callus response and fresh weight in both hypocotyl and internode explants. B_5 was intermediate, whereas WPM supported lower fresh weights in the present comparison. The differences indicate that basal-medium formulation influenced callus development under the conditions tested. MS medium is widely used for plant tissue culture and provided the most favourable response among the three formulations evaluated for these seedling explants. The present results are specific to the explant source, PGR regime and culture conditions used; they do not establish that MS medium is universally superior for all *E. variegata* tissues or for later stages of regeneration.

4.3 PGR-dependent callus responses

Among the individual PGRs, IBA at 2.0 mg L^{-1} produced the highest recorded hypocotyl response (90%). IAA, NAA and picloram produced intermediate responses, while dicamba and 2,4-D produced lower percentages in the summarized comparison. Callus colour and texture also varied among treatments, demonstrating that response percentage alone does not describe callus characteristics. The data indicate that the auxin response was concentration- and explant-dependent.

The BAP–NAA treatments also produced high callus frequencies. The maximum response of 90% was recorded with 5.0 mg L^{-1} BAP plus 0.5 mg L^{-1} NAA, and the callus was described as green and friable. This combination therefore supported callus induction under the conditions tested. It should not, however, be interpreted as an optimum for shoot organogenesis because the present experiments did not quantify shoot-bud formation or plantlet regeneration from these calli.

The addition of $AgNO_3$ to BAP-containing media resulted in light-green, friable callus, with response percentages of 60–75% across the tested concentrations. Other combinations containing IAA, IBA, 2,4-D, casein hydrolysate or coconut milk produced variable responses. The available records describe callus frequency and morphology but do not provide comparative quantitative data on subsequent morphogenesis for these treatments.

4.4 Callus induction in relation to regeneration studies in *Erythrina*

Earlier studies in *E. variegata* have documented shoot formation, organogenesis and rooting under different in vitro conditions. Shasthree *et al*. [9] reported rooting from callus cultures derived from seedling explants and noted that rhizogenesis was more frequent than caulogenesis under the conditions they examined. Shasthree *et al*. [10] described plantlet regeneration by organogenesis, while Javed and Anis [3] established a nodal-explant micropropagation protocol. These studies demonstrate that regeneration outcomes depend on the source and developmental state of the explant and on the PGR sequence applied. The present work focuses on callus induction and identifies responsive explant–medium–PGR combinations.

Callus-mediated regeneration in other woody legumes, such as *Dalbergia latifolia*, has involved sequential changes in cytokinin and auxin balance [8]. This provides a useful comparative context for future studies, but the regeneration response of *E. variegata* must be determined experimentally rather than inferred from another species.

5. Conclusion

Seedling hypocotyls were the most responsive explants for callus induction in *Erythrina variegata* among the explants and basal media evaluated. MS medium supplemented with 2.0 mg

L^{-1} IBA produced 90% response and the highest recorded fresh callus weight (1060 ± 437.0 mg). A combination of 5.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA also produced 90% response, with green, friable callus. These results identify effective conditions for callus production from seedling-derived hypocotyls. The experiments document callus induction.

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Author Contributions
Sowghandika: research planning, methodology, tissue-culture experiments and initial manuscript preparation. Padma: subculture methodology, protocol standardization and manuscript preparation. Raja K Swamy: manuscript organization, editing and preparation. Madhavi: manuscript preparation and review.

Conflict of Interest
The authors declare that they have no conflict of interest related to this research or manuscript.

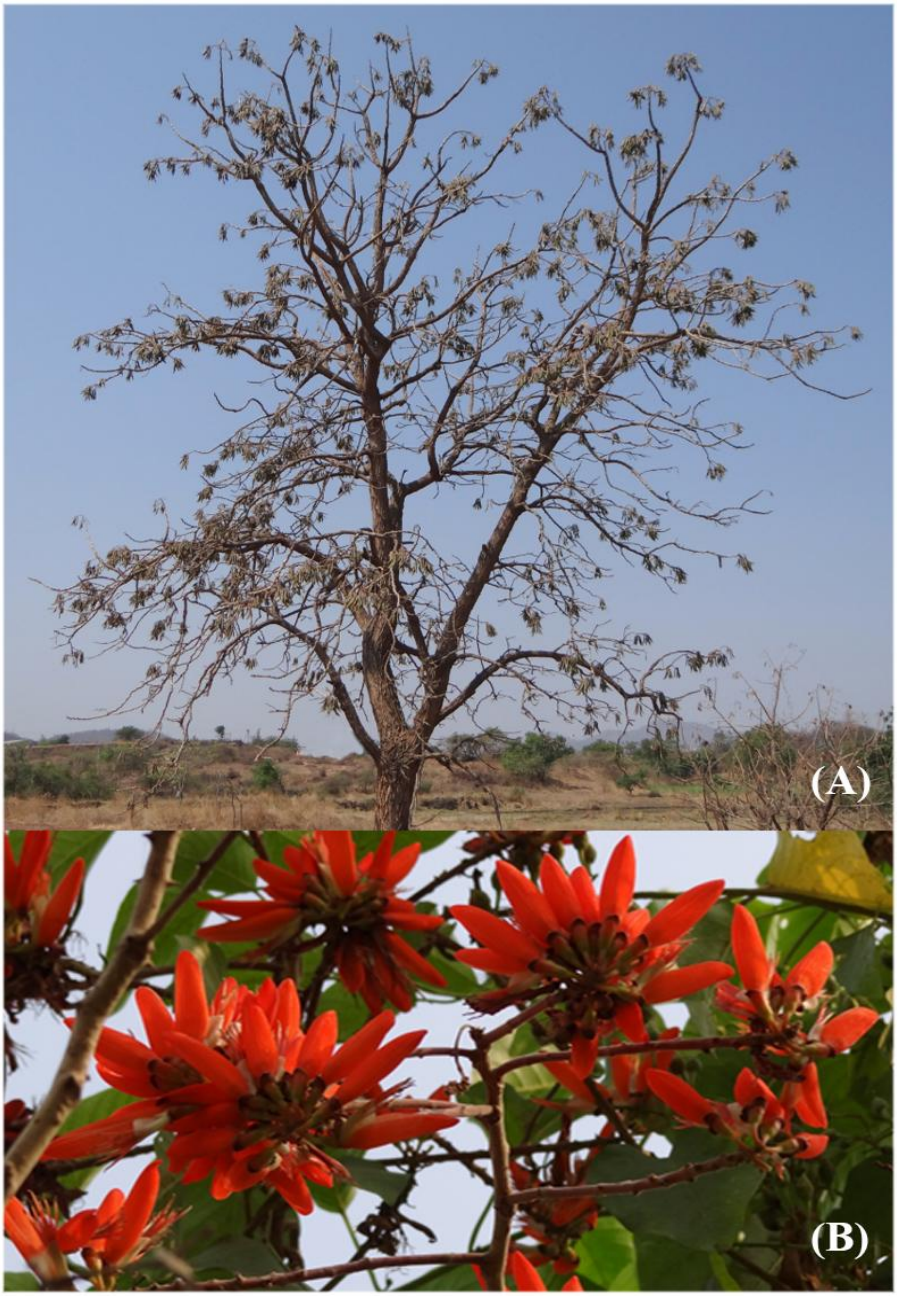


Plate 1: *Erythrina variegata* at Bakarapet, Andhra Pradesh: (A) fruiting tree, photographed in May; (B) flowers, photographed in March.

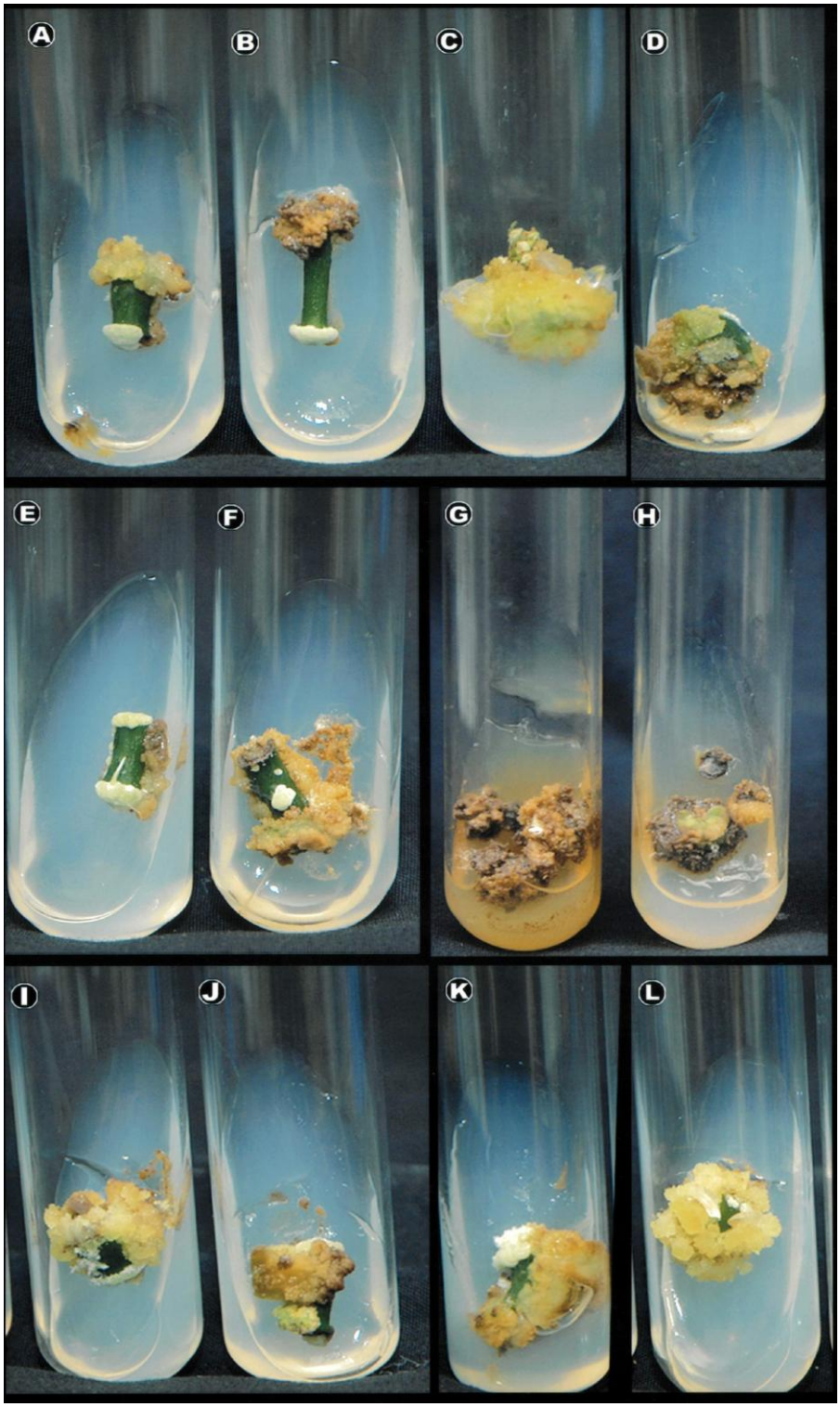


Plate 2: Callus induction from hypocotyl and internode explants of *Erythrina variegata* on MS medium supplemented with different plant growth regulators: (A) callus proliferation on Gamborg B_5 medium from a hypocotyl explant; (B) callus proliferation on MS medium from an internode explant; (C, D) callus proliferation from hypocotyl explants on MS medium supplemented with 2.0 mg L^{-1} IBA; (E) callus induction on MS medium supplemented with 1.0 mg L^{-1} picloram; (F) callus induction on MS medium supplemented with 1.0 mg L^{-1} dicamba; (G) callus induction on MS medium supplemented with 2.0 mg L^{-1} picloram; (H) callus induction on MS medium supplemented with 5.0 mg L^{-1} picloram; (I) callus induction on MS medium supplemented with 3.0 mg L^{-1} NAA; (J) callus induction on MS medium supplemented with 0.5 mg L^{-1} 2,4-D; (K) callus induction on MS medium supplemented with 2.0 mg L^{-1} dicamba; (L) callus induction on MS medium supplemented with 2.0 mg L^{-1} IBA.

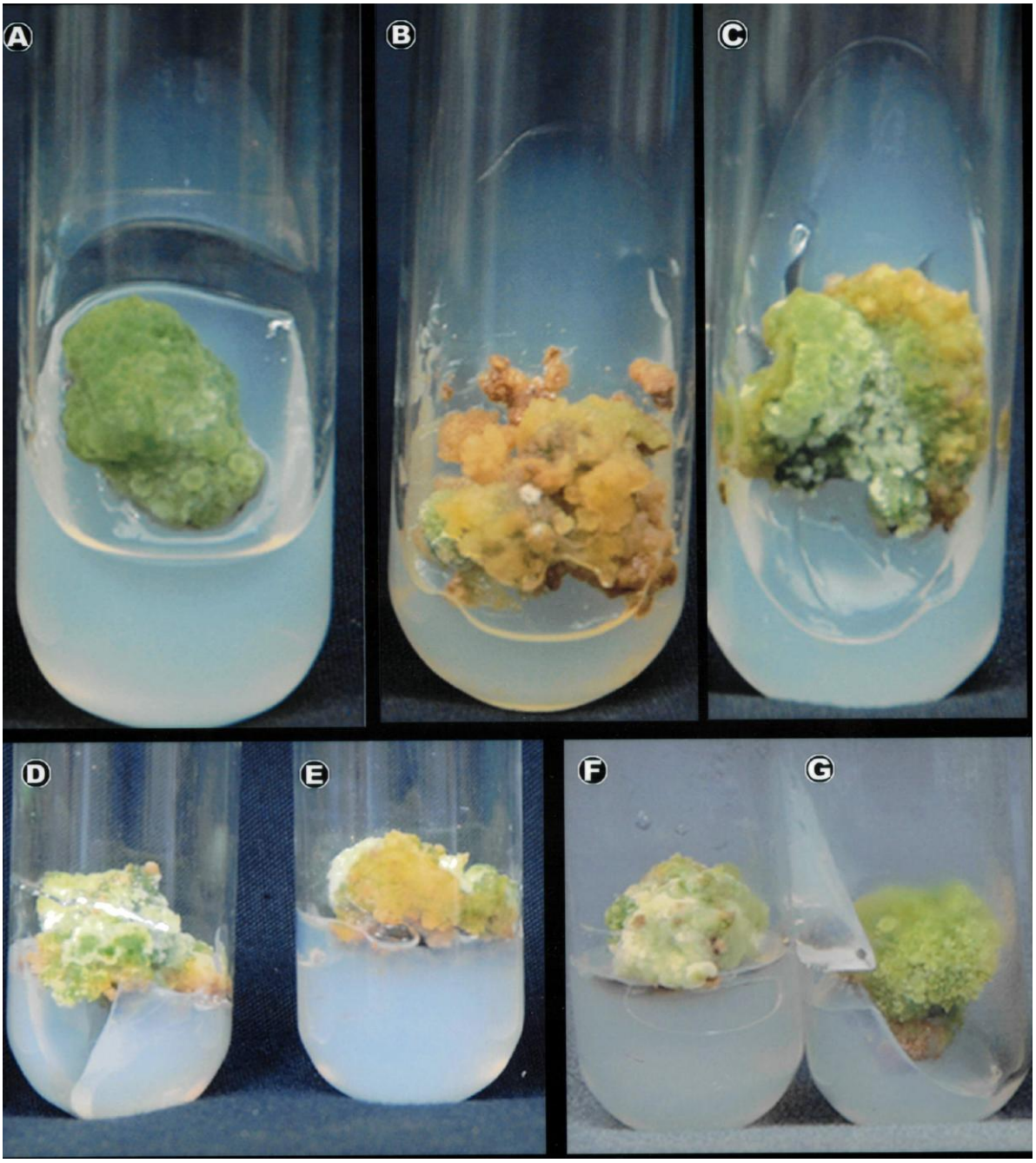


Plate 3: Callus induction from hypocotyl explants of *Erythrina variegata* on MS medium supplemented with BAP and selected growth additives: (A) 5.0 mg L^{-1} BAP; (B) 5.0 mg L^{-1} BAP + casein hydrolysate; (C) 5.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA; (D) 5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA; (E) 5.0 mg L^{-1} BAP + 0.2 mg L^{-1} $AgNO_3$; (F) 5.0 mg L^{-1} BAP + 0.2 mg L^{-1} IAA; (G) 5.0 mg L^{-1} BAP + 15% coconut milk.

References

[1] Carlini, I.C., Bertão, M.R. & Moraes, M.C., 'Evaluation of scarification and in vitro culture methods for callus production in the genus *Erythrina*', Acta Scientiarum. Biological Sciences, 47.1 (2025), e71360. https://doi.org/10.4025/actascibiolsci.v47i1.71360

[2] Gamborg, O.L., Miller, R.A. & Ojima, K., 'Nutrient requirements of suspension cultures of soybean root cells', Experimental Cell Research, 50 (1968), pp. 151–158.

[3] Javed, S.B. & Anis, M., 'Cobalt induced augmentation of in vitro morphogenic potential in *Erythrina variegata* L.: a multipurpose tree legume', Plant Cell, Tissue and Organ Culture, 120.2 (2015), pp. 463–474. https://doi.org/10.1007/s11240-014-0613-2

[4] Javed, S.B., Alatar, A.A., Anis, M. & Faisal, M., 'Synthetic seeds production and germination studies for short-term storage and long-distance transport of *Erythrina variegata* L.: a multipurpose tree legume',

Industrial Crops and Products, 105 (2017), pp. 41–46. https://doi.org/10.1016/j.indcrop.2017.04.053

[5] Javed, S.B., Alatar, A.A., Basahi, R., Anis, M., Faisal, M. & Husain, F.M., 'Copper induced suppression of systemic microbial contamination in *Erythrina variegata* L. during in vitro culture', Plant Cell, Tissue and Organ Culture, 128.2 (2017), pp. 249–258. https://doi.org/10.1007/s11240-016-1104-4

[6] Lloyd, G. & McCown, B.H., 'Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot-tip culture', Combined Proceedings of the International Plant Propagators' Society, 30 (1980), pp. 421–427.

[7] Murashige, T. & Skoog, F., 'A revised medium for rapid growth and bio assays with tobacco tissue cultures', Physiologia Plantarum, 15 (1962), pp. 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

[8] Sankara Rao, K., 'Plantlets from somatic callus tissue of the East Indian Rosewood (*Dalbergia latifolia* Roxb.)', Plant Cell Reports, 5.3 (1986), pp. 199–201. https://doi.org/10.1007/BF00269118

[9] Shasthree, T., Imran, M.A. & Mallaiah, B., 'In vitro rooting from callus cultures derived from seedling explants of *Erythrina variegata* L.', Current Trends in Biotechnology and Pharmacy, 3.4 (2009), pp. 447–452.

[10] Shasthree, T., Madhavi, S. & Mallaiah, B., 'Regeneration of plantlets in *Erythrina variegata* L. by organogenesis', Research Journal of Biotechnology, 4.3 (2009), pp. 30–37.

[11] Shasthree, T., Savitha, R., Venkateshwarlu, K. & Mallaiah, B., 'In vitro propagation of the leguminous tree *Erythrina variegata* L. via multiple shoot formation', Plant Cell Biotechnology and Molecular Biology, 10.1–2 (2009), pp. 75–78.